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Note

Comparative Study of the Proliferative Activity of Serous- and Mucous-type Acinar Cells in Developing Mongolian Gerbil Mixed Salivary Glands

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We estimated the proliferative activity of serous and mucous acinar cells in mixed-type salivary glands of sucking Mongolian gerbils, using immunohistochemistry with proliferating cell nuclear antigen (PCNA) and *in vivo* labeling of S-phase cells with the thymidine analog bromodeoxyuridine (BrdU). In both sublingual and Weber's (postlingual) salivary glands, acinar secretory cells showing immunoreactivity for anti-PCNA and anti-BrdU were restricted to serous-type cells that were also positive for a serous component, lysozyme. Some less-differentiated cells found in the serous demilunes or acini, which were negative for lysozyme, also showed proliferative activity, while mucous-type cells never did. These results suggest that in the histogenesis of the mixed gland endpieces, the serous-type cells include immature, differentiating cells, while the mucous-type cells consist of only mature, well-differentiated cells.

Key words: Proliferative activity, Serous cell, Mucous cell, Mixed Salivary Glands, Mongolian gerbil

I. Introduction

Two types of secretory cells, i.e., serous and mucous cells, are distinguished in the acini of mixed-type salivary glands. As a rule, the serous cells are located at the terminal portions, and the mucous cells are arranged between the serous cells and intercalated duct cells. In previous reports on human sublingual and labial glands, we suggested that these two different phenotypes are expressed during the maturation of a single cell lineage. That is, the acinar secretory cells of these mixed glands appear to have a serial morphological relationship, and immature secretory cells are restricted to lysozyme-expressing, serous-type cells [4, 5]. This implies that the serous-type cells proliferate at the terminal portion, migrate toward the proximal portion, and then transdifferentiate into mucous cells. This hypothesis is contrary to current theories concerning salivary gland histogenesis, such as the semipluripotential bicellular reserve cell hypothesis [3], in which acinar cells arise from progenitor cells in the intercalated ducts. As far as we know, there are no reports on the difference in the proliferative activities of serous- and mucous-type acinar cells in mixed salivary glands. Therefore, this study explored this as one step towards verifying our hypothesis.

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II. Materials and Methods

The sublingual and Weber's (postlingual) salivary glands of sucking Mongolian gerbils (0, 1, 6, 7, 11 and 14 days after birth) were examined. For each age group, three animals were used. Proliferating cells were detected by immunocytochemistry for endogenous proliferating cell nuclear antigen (PCNA) and *in vivo* labeled bromodeoxyuridine (BrdU). BrdU was injected intraperitoneally (0.05 mg/g body weight) 2 hr before sacrifice. After the animals

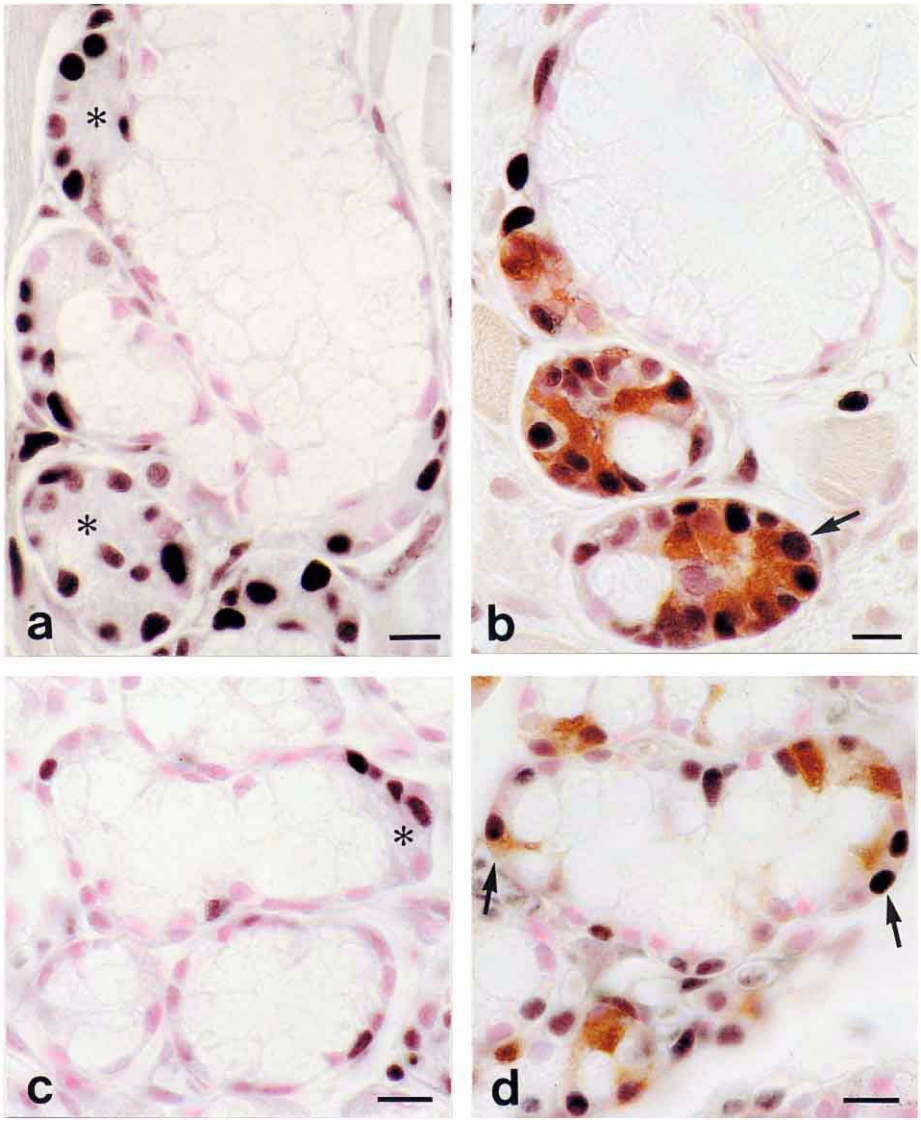

Fig. 1. Light micrographs of sucking Mongolian gerbil Weber's postlingual (**a, b: a.** 11 day, **b.** 6 day) and sublingual (**c, d: c.** 7 day, **d.** 1 day) salivary glands, immunostained with anti-PCNA and counterstained with nuclear fast red. Sections **b** and **d** are double-stained with anti-lysozyme. PCNA-positive cells (with nuclei colored black) are found over the serous acinar or demilunar portion (asterisks). Many of the lysozyme-positive cells (with cytoplasm colored brown) show reactivity for anti-PCNA (arrows). ×720 (**a, b**), ×670 (**c, d**). Bars=50 μm.

were anesthetized by an intraperitoneal injection of sodium pentobarbital, specimens were removed, fixed in 4% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.4), and embedded in paraffin. Four-micrometer-thick sections were cut and mounted on glass slides, then deparaffinized in xylene and rehydrated. After blocking endogenous peroxidase activity with 0.3% H_2O_2 in methanol for 30 min, the sections were incubated with either anti-PCNA mouse monoclonal antibody (PC 10) at a 1:10 dilution, or nuclease/anti-BrdU mouse monoclonal antibody for 1 hr at room temperature. Then, they were incubated with peroxidase-labeled anti-mouse IgG2a for 30 min. Finally, the antibody binding sites were visualized as a black reaction product by applying 0.05% 3,3'-diaminobenzidine (DAB), 0.01% nickel chloride, and 0.01% H_2O_2 in phosphate buffer for 8 min, and then counterstaining with nuclear fast red. The BrdU, nuclease/anti-BrdU, and peroxidase-labeled anti-mouse IgG2a were the reagents from a cell proliferation kit (Amersham Pharmacia Biotech, Tokyo, Japan), and the anti-PCNA was purchased from PROGEN Biotechnik GmbH (Heidelberg, Germany). Following the immunocytochemistry for PCNA or BrdU, some sections were also stained for lysozyme to identify the serous-type secretory cells. After incubation with anti-lysozyme (A 0099; DAKO, Glostrup, Denmark) for 1 hr at room temperature, the slides were incubated with peroxidase-labeled anti-rabbit IgG (Histofine Simple Stain MAX PO; Nichirei, Tokyo, Japan) for 30 min at room temperature. Then, the sections were colored brown by incubation with a DAB solution (the processing solution used above without nickel chloride).

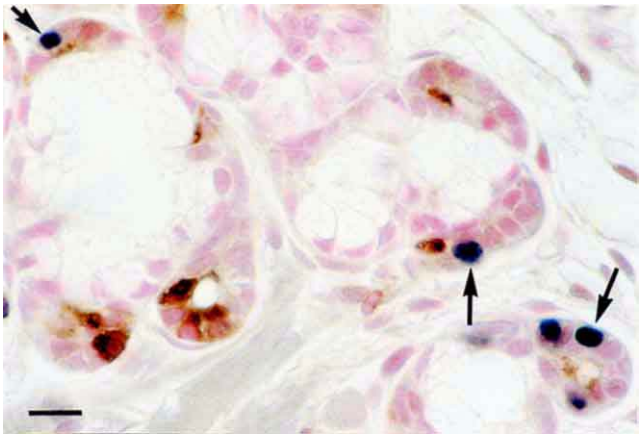

Fig. 2. Light micrographs of sublingual salivary glands of 6-day-old Mongolian gerbil, immunostained with anti-BrdU and anti-lysozyme, and counterstained with nuclear fast red. BrdU-positive cells (with nuclei colored black) are found on the serous acinar or demilunar portion (arrows) containing lysozyme-positive cells (with cytoplasm colored brown). ×670. Bars=50 μm.

Control reactions were carried out by omitting the primary antisera.

III. Results and Discussion

The secretory ends of the sublingual and Weber's salivary glands in suckling gerbils already appeared to be typical mixed glands; two types of acinar cells could be distinguished: serous- and mucous-type cells. In the secretory ends of both glands, PCNA-immunoreactivity was detected mostly in the nuclei of the serous-type secretory cells located in the acinar or demilunar portion. Some cells that appeared less differentiated, with few or no secretory granules, and some basal cells (probably myoepithelial cells) were also positive for anti-PCNA, but the mucous-type secretory cells never showed reactivity (Fig. 1a, c). Many of the PCNA-positive cells revealed anti-lysozyme reactivity, confirming that they were serous-type cells. Although cells that were lysozyme-negative, but PCNA-positive also existed, they were located at the serous acini or demilunes, and basal portion, and never showed the morphological appearance of mucous-type cells. Lysozyme-positive cells were not always positive for anti-PCNA (Fig. 1b, d). The localization of BrdU-positive secretory cells was similar to that of the PCNA-positive cells, although their labeling frequency was very low (Fig. 2).

These results clearly indicate that the secretory cells with proliferative activity in the developing gerbil mixed salivary gland are the serous types. This suggests that the serous-type cells include differentiating, immature cells, and that the mucous-type cells have no ability to divide, and are highly differentiated, mature cells. This does not necessarily imply that the serous cells transdifferentiate into mucous cells, because the mucous cells may differentiate from intercalated duct cells, which are classically believed to be their progenitors. However, it provides some support to our hypothesis described above [4, 5]. Studies of developmental changes in the ultrastructure and peroxidase activity in rat sublingual and submandibular gland acinar cells also suggest that the serous cells differentiate into seromucous or mucous cells during histogenesis of the endpieces [6, 7]. In a different interpretation of an immunocytochemical study of the expression pattern of several secretory proteins in developing rat sublingual glands, Wolff *et al.* [9] suggested that the serous and mucous cells differentiate via separate cell lineages. It is necessary to examine the relationship between their expression pattern and proliferative activity of the secretory cells.

Many studies have proved that all salivary gland cell types are capable of proliferation, including secretory cells [e.g., see 1, 2, 8]. Redman [8] made an important suggestion, that the acinar cell is the primary candidate as the stem cell of the rat parotid gland and must migrate and differentiate into ducts. However, these investigations have been restricted to "serous-type" glands, and there are no reports on the differences in the proliferative activity of serous and mucous secretory cells in mixed glands. Therefore, this report focused on their proliferative activity. To examine our hypothesis and the mechanism of the histogenesis of the endpiece of mixed glands, we plan extensive studies in the future, including studies of the duct system.

IV. References

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